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CCK-8S Facilitates 5-HT Release in the Rat Hypothalamus

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VOIGT, J.-P., R. SOHR AND H. FINK. *CCK-8S facilitates 5-HT release in the rat hypothalamus.* PHARMACOL BIOCHEM BEHAV **59**(1) 179–182, 1998.—The effects of the neurotransmitter serotonin (5-HT) and the neuropeptide cholecystokinin (CCK) on food intake are well established. Based on pharmacological studies, an interactive model for 5-HT and CCK was proposed. The present microdialysis study was aimed to provide neurochemical evidence for a facilitatory effect of CCK-8S on 5-HT release in the lateral hypothalamus under in vivo conditions. The results indicate an increase of extracellular hypothalamic 5-HT both during food intake in previously food-deprived rats and also after systemic administration of 8 μg/kg and 40 μg/kg CCK-8s in food-deprived rats. The results show that peripherally administered CCK-8s induces central serotonergic effects, possibly related to feeding. © 1998 Elsevier Science Inc.

Rat Hypothalamus Serotonin CCK Microdialysis Feeding

FOOD intake is an activity in animals and humans essential for survival. Therefore, it appears reasonable that different and perhaps partially redundant physiological systems have evolved to ensure the function of the whole system under different conditions. The feeding system is of complex nature regarding both structure and function. Many classical neurotransmitters as well as peptides are capable of modulating feeding behavior and food intake. It is necessary that the feeding system as a whole requires a coordination and interactions of its components. However, the underlying mechanisms of such interactions are poorly understood so far in detail.

For example, the effects of the neurotransmitter serotonin (5-HT) and the neuropeptide cholecystokinin (CCK) on food intake are well established (3,11,20). Based on pharmacological studies, an interactive model for 5-HT and CCK was proposed. This model suggests interdependent parallel systems of CCK and 5-HT both of which have to be activated to achieve an appropriate satiety effect (6–8). CCK exerts its satiating action in the periphery and at different sites within the brain including the hypothalamus (17), a brain region, involved in the central regulation of feeding. Electrophysiological studies demonstrated excitatory effects of CCK in the hypothalamus (4). Evidence was found that CCK-induced anorexia is 5-HT dependent (23). Microdialysis studies showed an increase in extracellular 5-HT during feeding in the hypothalamus (18,19). During nocturnal feeding, an increased 5-HT metabolism was observed in the lateral hypothalamus (LHA). This was paralleled by an increasing activity of 40% of LHA neurons, 23% decreased, and 37% of the neurons showed no change (1). The role for 5-HT in the control of food intake by the LHA was recently reviewed (2). Because the LHA is innervated by serotonergic neurons originating in the raphe (14), a facilitatory effect on the 5-HT system of CCK may also result in an increase in extracellular 5-HT in the LHA as observed during feeding. In an earlier study, serotonergic changes in different hypothalamic regions but not in the LHA, have been described after CCK administration (15). However, the authors measured the tissue content of 5-HT instead of release, questioning the behavioral significance of the data.

The present study was aimed, therefore, to provide neurochemical evidence for a facilitatory effect of CCK-8S on 5-HT release in the LHA under in vivo conditions. For this purpose, we administered anorectic doses of CCK-8S peripherally and measured 5-HT release in the LHA.

METHOD

Animals

Experiments were carried out in young adult male Wistar rats (Winkelmann, Germany) weighing 220–280 g. The rats

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were kept under standardized conditions with an artificial 12-h dark–light cycle (lights on 0700–1900 h). They had free access to a standard rat laboratory diet (Altromin 1326) and water.

Surgery

Rats were anesthetized with sodium pentobarbitone (45 mg/kg IP) and placed in a stereotaxic frame to allow the implantation of a microdialysis guide canula (CMA, Sweden). The coordinates were AP −2.5 mm, L 1.5 mm, from bregma, and 5.5 mm from the skull surface, according to the atlas of Paxinos and Watson (16). The guide canula was fixed to the skull with stainless steel screws and cold curing resin (Technovit, Kulzer, Germany). Rats were allowed at least 1 week for postoperative recovery before starting the microdialysis experiments. During this time the rats were kept individually in cylindrical cages allowing also to perform the microdialysis experiments.

Microdialysis

The microdialysis membranes (CMA 10) were 3 mm long, with an outer diameter of 0.5 mm and 20,000 molecular weight cutoff. According to our in vitro calibration test, the relative recovery was around 20% for 5-HT. The microdialysis probe was perfused (1 μl/min) with artificial CSF (125 mM NaCl, 2.5 mM KCl, 27 mM $NaHCO_3$, 0.5 mM NaH_2PO_4 H_2O, 2.4 mM Na_2HPO_4 $2H_2O$, 0.5 mM Na_2SO_4, 1mM $MgCl_2$ 6 H_2O, 1 mM $CaCl_2$ $2H_2O$, pH 7.4). The flow rate (1 μl/min) allowed the collection of 20 μl samples every 20 min into microvials.

Analysis of Dialysates

Dialysates were analyzed by reverse-phase high-performance liquid chromatography with electrochemical detection (HPLC-EC). Samples were injected directly into a valve with a 5-μl loop (Rheodyne, USA). The sample was separated by a 100 mm (RP 18, 3 μm, i.d. 1 mm; BAS) column. The mobile phase contained 0.15 mM NaH_2PO_4, 1 mM EDTA, 0.23 mM OSA, 4% isopropanol at pH 3.7. Mobile phase was delivered by a pump (Gynkotek, Germany) with external pulse dampener at flow rate of approximately 50 μl/min. Serotonin was oxidized at 0.650 V (Antec VT03 electrochemical cell and EP-30 electrochemical detector, Gynkotek, Germany).

Drugs

Cholecystokinin octapeptide (CCK-8, sulfated form, 8 μg/kg and 40 μg/kg) was freshly dissolved in 0.9% saline and injected IP. All injections took place after collecting three 20-min baseline samples.

CCK-8S was synthesized by Dr. P. Henklein, Institute of Biochemistry, Humboldt-University, Berlin, Germany.

Experimental Design

The dialysis probe was inserted through the guide canula at 1600 h the day before the experiment. At the same time, all food was removed from the animal cage. Water was always available. Overnight perfusion was performed with a flow rate of 0.5 μl/min. On the day of the experiment, the flow rate was increased to 1 μl/min allowing stable levels for 5-HT to be reached approximately within 2 h thereafter.

Experiment I

After collecting three 20-min baseline samples, a 1-h test meal of the same animal diet the rat was accustomed to was provided. Microdialysis sampling was continued for 60 min after finishing the test meal. In the control group, microdialysis was performed in the same way, but no test meal was provided.

Experiment II

Eighteen-hour food-deprived rats were subjected to the same procedure as described above, however, no test meal was provided. After collecting three 20-min baseline samples, 8 μg/kg or 40 μg/kg CCK-8S were injected IP. Food-deprived control rats were injected with saline. Six postinjection samples were then collected.

Potassium-Induced 5-HT Release

To check on the neuronal origin of the detected 5-HT, release was stimulated by perfusing the lateral hypothalamus with CSF containing 50 mM KCl for 5 min. This procedure increased 5-HT release to approximately 600%.

Histology

At the end of the microdialysis experiment, the brain was removed and frozen on the stage of a cryomicrotome. The brain was cut into serial coronal sections at 30 μm. The localization of the microdialysis probe was verified under a microscope by an observer unaware of the experimental data (see Fig. 3).

Statistical Analysis

Baseline release rates of 5-HT showed interindividual differences. Therefore, data from each animal were expressed as percentages. Data from three dialysates before treatment or feeding, respectively, were averaged and the mean set as 100%; all other individual values were calculated accordingly. Experiment I: means obtained for each 20-min sample from the experimental group and the control were compared by using the Student's *t*-test, or, where normal distribution failed, by the nonparametric Mann–Whitney *U*-test. Experiment II: means obtained for each 20-min sample from the experimental groups and the control were compared by ANOVA, or where normal distribution failed, by nonparametric Kruskal–Wallis-test, followed by Dunn's test. A probability level of $p < 0.05$ was regarded as significant.

RESULTS

Effect of Food Intake on 5-HT Release

In Experiment I, 5-HT release increased significantly to 164% within 40 min after providing food ($U = 6$, $p = 0.0011$). Compared to still food-deprived controls, 5-HT release remained significantly higher in the feeding group 20 min (131% of baseline, $t = 3.66$, $p = 0.0023$), 40 min (128% of baseline, $U = 12.5$, $p = 0.0090$), and 60 min (118% of baseline, $t = 3.22$, $p = 0.0123$) after removing food from the cage (Fig. 1). The average food intake in this group was 4.68 ± 0.85 g/1 h ($n = 12$).

Effect of CCK-8S on 5-HT Release

In the second experiment, extracellular 5-HT increased significantly to 145% 20 min after intraperitoneal administration of 8 μg/kg CCK and to 161% after 40 μg/kg CCK ($H = 8.72$, $p = 0.0128$). 5-HT release reached a significant maximum of 173% 40 min following 40 μg/kg CCK ($H = 10.1$, $p =$

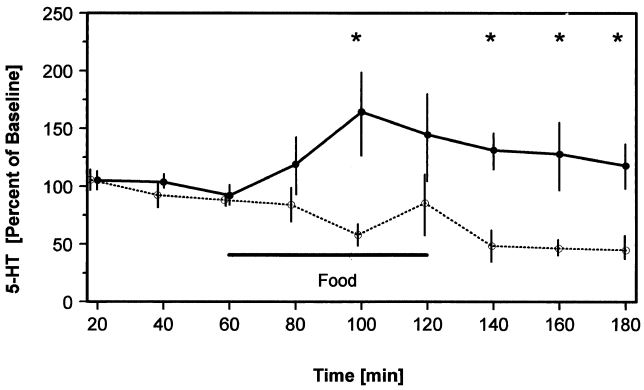

FIG. 1. Effect of food intake (1 h-test meal) on extracellular serotonin (5-HT) in previously food-deprived rats ($n = 12$) as compared to controls (dotted line, $n = 9$). Data points are expressed as percent of baseline (mean of the first three samples set 100%). Mean ± SEM. $*p < 0.05$ vs. control.

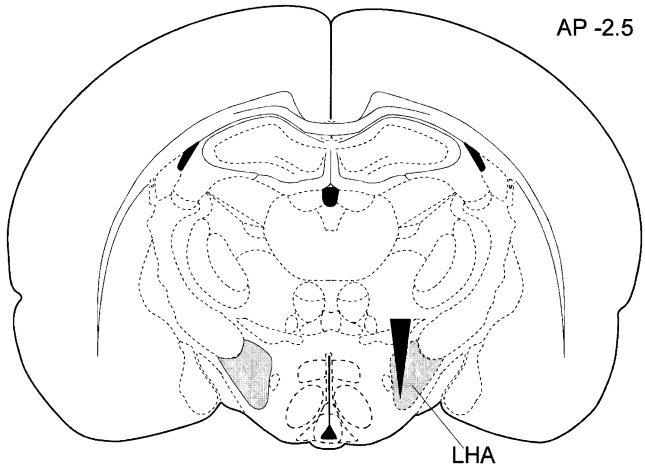

FIG. 3. Schematic drawing based on Paxinos and Watson (16) of the LHA (shadowed areas). The arrow indicates the tip of the dialysis probe.

0.0063). 5-HT release was still significantly higher 80 min following 8 μg/kg CCK ($H = 7.8, p = 0.0202$) and 120 min after 8 and 40 μg/kg CCK ($H = 8.5, p = 0.0143$) when compared with the control group (Fig. 2).

In both of the control groups, however, a slight but steady decrease of 5-HT over the experimental period was observed . Injection of saline had no effect on extracellular 5-HT release in food-deprived controls (Figs. 1 and 2).

DISCUSSION

In the present study, an increase in extracellular hypothalamic 5-HT was observed both during food intake in previously food-deprived rats and after systemic administration of CCK-8S in food-deprived rats. The first observation, an increase in 5-HT during feeding, confirms earlier results reporting an increase of approximately the same magnitude in microdialysates from the LHA (Fig. 3). Despite the wide acceptance that central serotonergic pathways participate in regulating food intake, the particular sites involved still need to be identified (21). The present data are in line with earlier microdialysis studies suggesting the involvement of the LHA in the central serotonergic regulation of feeding (18,19). However, other hypothalamic sites may be involved also, but possible interactions between these sites are still poorly understood. For example, the earlier distinction between feeding center (LHA) and satiety center (VMH) appears as too simplified in the light of present knowledge (13). The neuropeptide CCK, and in particular the sulfated octapeptide CCK-8S, has an hypophagic effect after systemic application. Additionally, several central sites of action have been found for the peptide including the LHA and the nucleus tractus solitarius (NTS) of the brain stem (5,17).

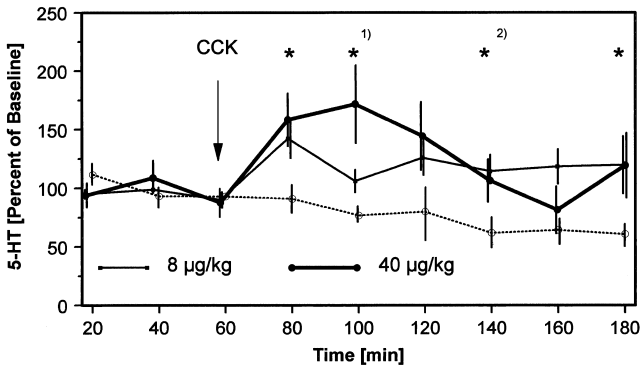

FIG. 2. Effect of CCK-8S (8 μg/kg, $n = 6$, and 40 μg/kg, $n = 9$; IP) on extracellular serotonin (5-HT) in food-deprived rats compared to saline-treated controls (dotted line, n = 9). Data points are expressed as percent of baseline (mean of the first three samples set 100%) Mean ± SEM. $*p < 0.05$ vs. control ([1])40 μg/kg only, [2])8 μg/kg only).

The predictive value of the interactive model for CCK and 5-HT in the control of feeding should allow to detect changes in serotonergic activity dependent on CCK and vice versa (6–8). In vitro experiments revealed stimulating effects of CCK on 5-HT neurons in hypothalamic structures (4). We (24) and others [(8), for review] found pharmacological evidence for 5-HT–CCK interactions in the control of feeding. Therefore, we expected to obtain also neurochemical evidence for a facilitatory effect of CCK-8S on 5-HT release in the LHA under in vivo conditions.

The results of our present microdialysis study confirm this assumption; however, some aspects of the experimental design need further consideration. Both of the CCK-8S doses administered had hypophagic effects in previous studies in our laboratory (24,25). In water-deprived rats, however, the highest dose (40 μg/kg CCK-8S) did not affect water intake despite sedative effects in the open field (25). Thus, the strong hypophagic effect after 40 μg/kg CCK-8S could not be attributed to sedative effects. Therefore, even a high dose of CCK-8S like 40 μg/kg may exert functionally specific effects on food intake.

The particular pathway underlying the effect of CCK upon the serotonergic system remains to be elucidated. Hypophagic effects of peripherally administered CCK are thought to be relayed to central sites by the vagus (9,22). Another possibility is, that exogenous CCK may directly reach the brain stem where the blood–brain barrier is more permeable. CCK_A receptors are located in the NTS of the brainstem (12). This assumption is in keeping with reports demonstrating an excita-

tory action of CCK-8 on the NTS in the brainstem (5). From the NTS the signal may be relayed to the hypothalamus (10).

The time course of 5-HT release observed in our microdialysis study is parallel to the time course of the hypophagic action of CCK-8S found in feeding experiments. In conclusion, the present study supports the hypothesis that peripherally administered CCK-8S is capable of facilitating hypothalamic 5-HT release comparable to the effect observed during feeding.

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